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Small and transient response of winter soil respiration and microbial communities to altered snow depth in a mid-temperate forest

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ABSTRACT

Global climate change is altering snow depth in winter, which could significantly affect soil respiration and microbial communities. However, belowground responses are still uncertain as they depend on the thermal effects on soils, the acclimation of soil microbial communities and ecosystem type. Here, we conducted a snow manipulation experiment including 50% removal of snowpack (mean snow depth after treatment was 3.1 ± 0.7 cm), ambient snow (mean snow depth was 6.3 ± 0.7 cm), and 50% increase of snowpack (mean snow depth after treatment was 9.6 ± 1.5 cm) to explore the effects of altered snow depth on winter soil respiration and microbial communities in a mid-latitude plantation forest with continental climate with dry winters. Winter soil CO_2 effluxes varied from 0.09 to 0.84 μmol $m^{-2} s^{-1}$ with a mean of 0.32 ± 0.07 μmol $m^{-2} s^{-1}$. The cumulative soil CO_2 effluxes from 11 December 2014 to 21 March 2015 were 27.3 ± 1.1, 26.5 ± 2.1, and 29.5 ± 1.3 g C m^{-2} under reduced, ambient and added snowpack, which corresponded to 5.7 ± 0.2%, 5.5 ± 0.3%, and 5.8 ± 0.1% of the annual soil CO_2 effluxes, respectively. Our one-year observation results suggested that although snow reduction decreased soil temperature, microbial biomass carbon (MBC) and soil respiration did not change, suggesting microbial adaptation to cold conditions between −4 °C and −1 °C. In contrast, snow addition increased soil temperature, MBC, and soil respiration. Microbial community structure (F/B, ratio of fungi to bacteria) was also changed and soil enzymatic (β-glucosidase) activities increased under snow addition. However, these effects were short-lived and disappeared when soil temperature did not differ between the addition and control plots at the 14th day after treatment. These results indicated that the responses of soil microbial communities and respiratory activities to changing soil temperature were rapid and the response of soil respiration to snow addition was transient. Consequently, altered snow depth did not affect cumulative soil CO_2 effluxes. Our study suggests that wintertime soil respiration rates are generally low and snow manipulation has minor effects on soil CO_2 efflux, soil temperature (the determinant driver of wintertime soil CO_2 efflux) and soil microbial biomass at our site.

1. Introduction

Climate projections indicate that global mean annual air temperature will increase 2–5 °C by year 2100 compared with preindustrial values (Rogelj et al., 2012; IPCC, 2013). Global warming is predicted to significantly change the depth of snow cover (Qin et al., 2006; IPCC, 2007; Peng et al., 2010), especially in mid-latitude (N 30–60° and S 30–60°, non-alpine) terrestrial ecosystems (Henry, 2008; Kreyling and Henry, 2011). Changes in snowpack depth associated with climate change have been shown to profoundly impact the soil carbon cycle, especially soil respiration (Monson et al., 2006b; Nobrega and Grogan, 2007; Du et al., 2013; Durán et al., 2014). Because winter soil respiration makes an important contribution to annual carbon budgets by accounting for 3–50% of annual carbon emissions (Hubbard et al.,

Abbreviations: CO_2, Carbon dioxide; F/B, Ratio of fungi to bacteria; C, Carbon; N, Nitrogen; TDR, Time domain reflectometry; PVC, Polyvinyl chloride; SR, Soil respiration; Q_{10}, increased soil respiration rate per 10 °C increase in soil temperature; EOC, Extractable organic carbon; TDN, Total dissolved nitrogen; TOC, Total organic carbon; TN, Total nitrogen; MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen; PLFA, Phospholipid fatty acid; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; AM, Arbuscular mycorrhizal; EAAs, Extracellular enzymatic activities; PCA, Principal component analysis; RDA, Redundancy analysis

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2005; Mo et al., 2005; Monson et al., 2006a; Elberling, 2007; Schindlbacher et al., 2007; Liptzin et al., 2009; Wang et al., 2010; Shi et al., 2012; Schindlbacher et al., 2014; Shi et al., 2014), it is important to understand how winter soil respiration responds to altered snow depth.

The impacts of snow depth on soil respiration in winter mainly results from its regulation of soil temperature. Snow serves as an insulation layer, preventing soil freezing at cold winter temperatures (Brooks et al., 2011). A thicker snowpack could thus effectively decouple soil and air temperatures, generally resulting in higher soil respiration rates (Elberling, 2007; Nobrega and Grogan, 2007; Sullivan et al., 2008; Liptzin et al., 2009). A thinner snowpack could inhibit soil microbial activities by reducing soil temperature, which could lead to lower soil respiration rates (Brooks et al., 1997; Monson et al., 2006b; Blankinship and Hart, 2012; Liu et al., 2016). In addition, the reduction of snow pack may also increase the release of dissolved organic carbon and nitrogen from soil aggregates and fresh litter due to more pronounced daily variations in soil temperature (Joseph and Henry, 2008; Freppaz et al., 2012), which could enhance the substrate supply of soil microbial communities and thus potentially lead to higher soil respiration rates. In addition to soil temperature, snowpack could also affect soil water content, especially during snowmelt (Merbold et al., 2011; Shibata et al., 2013). Higher soil water content may stimulate soil microbial activities (Monson et al., 2006a; Aanderud et al., 2013), but it may also limit oxygen diffusion in the soil and thus, reduce respiratory activity in the soil (Yohannes et al., 2011). Therefore, although changes in snow cover depth have been frequently observed to affect soil C cycling, the underlying mechanism are still poorly understood and the magnitude is highly uncertain as it depends upon prevailing soil and snow conditions.

Soil microorganisms play a vital role in understanding the underlying mechanisms of the responses of winter soil respiration to altered snow depth (Lipson et al., 2002; Puissant et al., 2015). Soil microbial biomass might be higher in winter than in summer (Edwards et al., 2007; Puissant et al., 2015) and soil microbial community may be distinct during winter (Lipson et al., 2000). For example, Monson et al. (2006b) found that soil respiration was very sensitive to changes in snow depth because a unique soil microbial community exhibited exponential growth during winter beneath the snow. Increased snowpack depth could enhance microbial biomass (Buckeridge and Grogan, 2008), stimulate microbial activities (Schimel and Weintraub, 2003) and increase the ratio of fungi to bacteria (Robroek et al., 2013). On the contrary, decreased snowpack depth was found to diminish microbial biomass (Durán et al., 2013), constrain microbial activities (Tan et al., 2014), and alter microbial community composition (Zinger et al., 2009; Aanderud et al., 2013; Robroek et al., 2013). Examination of these changes of microbial characteristics could help better understand why soil respiration responded to altered snow depth.

A number of studies documented the effects of altered snowpack depth on the carbon cycle under climate change in the past decades (Li et al., 2016). Almost all studies were conducted in high latitude (N 60–90° and S 60–90°) and altitude regions (Grogan and Jonasson, 2006; Schimel et al., 2006; Elberling, 2007; Schindlbacher et al., 2007; Merbold et al., 2013; Semenchuk et al., 2016), while only few studies have been done in temperate regions (Groffman et al., 2006; Aanderud et al., 2013; Sanders-DeMott and Templer, 2017). Although temperate ecosystems are characterized by relatively short winters, they have a stronger variation in the amount of snow cover compared to arctic, boreal and alpine ecosystems (Peng et al., 2010; Aanderud et al., 2013; Yu et al., 2013). In China, snow depth showed a dynamic variation with an increasing trend during the period 1982–1998 and a decreasing trend during the period 1998–2005 across the entire temperate region (Yu et al., 2013). Due to the dry winter climate, the annual snow depth is normally low ranging from 0 cm to 10 cm in Northeast China (Peng et al., 2010; Yu et al., 2013). Here, we conducted a snow cover manipulation experiment to study the effects of altered snow depth on winter soil respiration in a mid-temperate forest plantation ecosystem in Northeast China. The objectives of our study were: (i) to assess how altered snow cover depth affected winter soil respiration rates; and (ii) to explore the underlying mechanisms from environmental, nutrient and microbiological perspectives.

2. Material and methods

2.1. Site description

The research site was located nearby Shenyang, Liaoning, northeast China (41°54′22″N, 123°35′48″E, elevation of 122 m). The research forest is a 20-year-old plantation forest, dominated by Larch (*Larix gmelinii (Rupr.) Kuzen.*). The mean annual temperature is 8.3 °C with a maximum monthly temperature of 24.8 °C in July and minimal monthly temperature of −10.5 °C in January, respectively. The mean annual precipitation is 726 mm and winter precipitation is 30 mm. The mean annual snow depth is approximately 6 cm from 1978 to 2006 (Chen et al., 2008). The annual snow depth was 63 ± 7 mm in the winter of 2015. The number of days with snow was 102 days from 11 December 2014 to 21 March 2015. Snowmelt started on 12 March 2015 and all snow had disappeared in all plots at on 21 March 2015. The minimum winter air temperature and minimum annual soil temperature were −21 °C and −2.9 °C, respectively. The site is on a south facing slope of 5°. The soil is sandy loam with a mull-type organic surface layer having a ~1 cm thick litter layer. The soil is classified as aquic Brown soil by Chinese Soil Classification (equivalent to Typic Haplaqualf by USDA Soil Taxonomy). The basic characteristics of the studied soils (0–5 cm) are as follows: soil texture: 45.0 ± 1.0% sand, 24.9 ± 0.4% silt, 30.1 ± 0.5% clay, pH: 4.7 ± 0.0, bulk density: 1.2 ± 0.0 g cm^{-3}, total C: 20.4 ± 0.8 g kg^{-1}, total N: 1.8 ± 0.1 g kg^{-1}, C:N: 12.0 ± 0.1.

2.2. Experimental design and snow manipulation

The experimental snow manipulation was laid out as a completely randomized design with a one-way treatment structure and the following three treatments: snow reduction (50% removal of snowpack, Reduction); control (ambient snow, Control) and snow addition (50% increase of snowpack, Addition). Each treatment had five replicates on 2.5 × 2.5 m plots with each containing one larch tree. The experimental plots were approximately 5 m apart from each other. A 0.5 m buffer was used around the edge of each plot to avoid disturbance and interactions among plots. In order to facilitate shoveling in the plots, the understory vegetation (30 herbs per square meter) was clipped in all plots. The snow manipulation was conducted immediately once each snowfall event exceeded 5 cm, namely on 18 December 2014, 28 January 2015 and 19 February 2015, respectively. On the snow-reduction plots, we left at least a 2.5 cm snow layer to avoid a disturbance of the forest floor and to keep the surface albedo. The treatment was done by gently shoveling the snow from the reduction treatment plot to the addition treatment plot using a shovel.

2.3. Microclimate monitoring

The forest air temperature (2 m above the ground) and soil temperature (5 cm depth) were recorded by buried Thermochron iButton (iButton DS1923-F5, Maxim Com.USA) in each plot. It recorded temperature every 2 h from 1 November 2014 to 1 April 2015. Snow depth was measured using a graduated stainless steel rod once a week. Soil volumetric water content (0–5 cm) was measured using TDR300 soil moisture probe (Spectrum Technologies Inc., Plainfield; IL, USA) (2 soil moisture probes in each plot).

2.4. CO_2 efflux, Q_{10} and soil sampling

Soil CO_2 efflux from the snow surface was measured using a Li-6400 soil CO_2 flux system (LI-COR INC., Lincon, NE, USA). Three polyvinyl chloride (PVC) collars (10.5 cm in diameter) were inserted into the snow surface in each plot and stabilized for 24 h before each CO_2 efflux measurement. We produced collars with different length for each measurement date so that the collars reached the soil surface and the height of the PVC collars reached 3 cm height from snow surface to above edge of the collar (Elberling, 2007; Wang et al., 2010). The distance of each PVC collar to the tree trunk was 50 cm. The Li-6400 was kept in a heated container to keep its temperature above freezing when air temperature was below −10 °C. Measurements were conducted once a week between 9:00 and 12:00 am. In the snow free period, soil CO_2 effluxes were also measured using the Li-6400 soil CO_2 flux system. PVC collars (10.5 cm in diameter) were inserted 3 cm into soil and the left 3 cm height from soil surface to above edge of the collar (Wang et al., 2010). The measurements were also made once a week between 9:00 and 12:00 am. The cumulative CO_2 flux during a given period was calculated by linearly interpolating between two measurements and integrating for the respective time period. The relationship between soil respiration and soil temperature was described using the following exponential function: $SR = \alpha \times e^{\beta t}$, where SR is soil respiration, t is soil temperature, α and β are constant coefficients. Q_{10} (increased soil respiration rate per 10 °C increase in soil temperature) was calculated as $Q_{10} = \exp(10 \times \beta)$.

Soils were sampled on 5 dates: the 6th day after the first snow manipulation (Dec. 24, 2014), the 7th day after the second snow manipulation (Feb. 4, 2015) and one week later (Feb. 11, 2015), the 7th day after the third snow manipulation (Feb. 26, 2015) and 8 days later (Mar. 6, 2015). Soil samples were randomly collected at three points in each plot using a soil auger (5 cm diameter, 0–5 cm depth), mixed homogeneously, stored in freezer boxes and then transported to the laboratory within 6 h. Fresh soil was passed through a 2.0 mm sieve and then stored in the refrigerator at −20 °C and 4 °C for microbial and chemical analysis, respectively.

2.5. Soil analysis

2.5.1. Basic soil characteristics

Soil pH was measured in de-ionized water (1:2.5 soil solution) using a pH meter (E-201-C, Leici, China). Soil gravimetric water content was measured by drying soil at 105 °C for 24 h. Bulk density was determined by the core method (Burke et al., 1986). Soil texture was measured by the pipette-sedimentation method (Gee and Bauder, 1986). Soil total C and total N were analyzed using an elemental analyzer (Vario Macro Elementar, Germany).

2.5.2. Soil inorganic N and extractable organic C and N

Soil inorganic N were extracted with 2 M KCl solution (fresh soil: 2 M KCl = 1:5, shaking for 1 h) and then measured using the indophenol-blue for ammonium and the phenol disulphonic acid colorimetry for nitrate, respectively (Lu, 1999). Extractable organic carbon (EOC) and total dissolved nitrogen (TDN) were extracted with 0.5 M K_2SO_4 solution (fresh soil: 0.5 M K_2SO_4 = 1:4, shaking for 1 h) and then determined using a TOC/TN analyzer (MultiN/C3100, Analytic Jena, Germany). The extractions were also analyzed for ammonium and nitrate using the indophenol-blue and phenol disulphonic acid colorimetry, respectively (Lu, 1999). Extractable organic nitrogen (EON) was calculated as EON = TDN- (ammonium + nitrate) (Ross et al., 2013).

2.5.3. Soil microbial biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987), where soils were extracted with 0.5 M K_2SO_4 (fresh soil: 0.5 M K_2SO_4 = 1:4, 200 rpm shaking for 1 h) before and after fumigation of soils with chloroform for 24 h. Extractable C and N in both fumigated and unfumigated extracts were analyzed using a TOC/TN analyzer (MultiN/C3100, Analytik Jena, Germany).

MBC and MBN were calculated as follows:

$$MBC = EC/0.45 \text{ and } MBN = EN/0.54$$

where EC is the difference between fumigated and unfumigated DOC content; EN is the difference between fumigated and unfumigated TDN content; 0.45 and 0.54 are two correction factors to account for incomplete extraction of MBC and MBN, respectively (Brookes et al., 1985; Vance et al., 1987).

2.5.4. Soil microbial community structure

Soil microbial phospholipid fatty acid (PLFA) composition was measured to study soil microbial community structure. PLFAs were analyzed using a modified Bligh-Dyer method (Wang et al., 2014). Total lipids were extracted from 4 g freeze-dried soil with a one-phase mixture (0.8:1:2 v/v/v) of phosphate buffer, chloroform and methanol, followed by a separation of phospholipids from total lipids using a silicic acid column. 19:0 methyl nonadecanoate (Sigma-Aldrich, USA) was added as an internal standard for quantifying the PLFAs. PLFAs were identified by the gas chromatograph (Agilent 7890, Agilent technologies, USA). We used the PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 as makers of Gram-positive bacteria (G^+), 16:1ω7c, 17:1ω8c, 18:1ω7c, cy17:0 and cy19:0 were used as markers for Gram-negative bacteria (G^-), 14:0, 15:0, 16:0 and 18:0 were used to represent general bacteria, 10Me16:0, 10Me17:0 and 10Me18:0 represented actinomycetes, 18:1ω9c and 18:2ω6,9c were used as indicators for fungi, 16:1ω5c was used as AM (Arbuscular mycorrhizal) fungi marker (Kaiser et al., 2010a; Kaiser et al., 2010b).

2.5.5. Soil extracellular enzymatic activities

Four soil extracellular enzymatic activities (EAAs) involved in carbon cycling were determined using fluorogenic substrates based on the method described by Marx et al. (2001). The enzymes α-glucosidase, β-glucosidase, cellobiohydrolase and xylosidase, were assayed with substrates of 4-methylumbelliferyl-α-D-glucosidase, 4-methylumbelliferyl-β-D-glucosidase, 4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-β-D-xylopyranoside, respectively. In order to ensure that the enzyme assays were under saturating substrate concentrations, the concentration of fluorogenic substrates was optimized before assaying soil enzyme activity (German et al., 2011). The optimized concentrations of fluorogenic substrates for α-glucosidase, β-glucosidase, cellobiohydrolase and xylosidase were 0.677, 0.677, 1.001, 0.617 mg L^{-1}, respectively. Briefly, soil suspensions were made with 2.00 g of fresh soil sieved at 2 mm into 100 ml sodium acetate buffer (pH = 5.0) and stirring on a magnetic stir plate for 1 min. 200 μL of soil suspensions and 50 μL of substrates were added into assay 96-well microplates. 200 μL of soil suspensions and 50 μL of sodium acetate buffer (pH = 5.0) were added into blank 96-well microplates. 200 μL of soil suspensions and 50 μL of 4-methylumbelliferyl were added into quench 96-well microplates. 200 μL of sodium acetate buffer (pH = 5.0) and 50 μL of 4-methylumbelliferyl were added into standard 96-well microplates. Then, 96-well microplates were incubated in the dark at 25 °C for 4 h. After incubation, 10 μL of 0.5 mol L^{-1} NaOH was immediately added to all 96-well microplates to terminate the assay. Finally, the fluorescence products were measured rapidly using a fluorimetric microplate reader (Synergy H1, BioTek, Winooski, VT), with excitation and emission wavelengths of 365 nm and 450 nm, respectively. Soil EEAs were calculated according to a method described by German et al. (2011).

2.6. Statistical analyses

The effects of altered snowpack depth on environmental factors, soil

nutrients, soil microbial characteristics and winter soil respiration were examined using repeated measures ANOVA and one-way ANOVA (Duncan's test at $P < 0.05$). The plot mean of soil moisture from the two probes and the plot mean of soil respiration from the three collars were used in the statistical analysis. A permutation test was used for testing the significance of soil microbial community structure and environmental variables (999 permutations). The relationships between winter soil respiration and environmental factors, soil nutrients, and soil microbial characteristics were analyzed by multiple stepwise regressions. Principal component analysis (PCA) and redundancy analysis (RDA) were performed in R Studio 3.2.0 (using the R packages vegan) to evaluate changes in microbial community composition under altered snow depth treatments, the other statistical analyses were carried out in SPSS 19.0 (SPSS Inc., IL, USA). Values were reported as mean ± standard errors throughout this paper.

3. Results

3.1. Snow depth and soil volumetric water content

Snow cover period lasted for 102 days from 11 December 2014 to 21 March 2015. There were three main snowfall events (18 December 2014, 28 January 2015, 19 February 2015). The maximum snow depth reached 143 mm on 4 February 2015. Snowmelt started in early March 2015. All snow has disappeared in all plots on 21 March 2015. Snow manipulation was conducted after each snowfall event and altered the mean snow depths during the whole study period from 63 ± 7 mm at ambient conditions to 31 ± 7 mm under snow reduction and 96 ± 15 mm under snow addition (Fig. 1a). Snow depth in the snow addition treatment was below 7 cm after the first manipulation and above 7 cm after the second and third manipulations (Fig. 1a).

Soil volumetric water content remained rather constant under all treatments during the snow cover period (from 5.1% to 9.0%) and strongly increased during the snowmelt period (from 9.1% to 24.3%, Fig. 1b). Snow manipulation clearly affected the soil volumetric water content with significantly higher values under Addition (16.1 ± 1.6%) than under Reduction (10.3 ± 1.4%) during the snowmelt period ($P < 0.05$), but not before.

3.2. Temperature and soil respiration

Soil temperature (5 cm depth) in all treatments had the same temporal pattern as air temperature (Fig. 2a and b). During the snow cover period, soil temperature varied from −4.5 to 0.5 °C and increased rapidly to 6.5 °C after snowmelt (Fig. 2b). Soil temperature differed significantly among treatments after the second and third snow manipulations with higher soil temperature under Addition and lower soil temperature under Reduction as compared to under Control ($P < 0.05$), but not before and during snowmelt (Table 1).

Temporal variations of CO_2 fluxes indicated winter soil respiration was highest during the snowmelt period (after 12 March 2015, Fig. 2c). Winter soil CO_2 fluxes were between 0.09 and 0.84 μmol $m^{-2} s^{-1}$ with a mean of 0.32 ± 0.07 μmol $m^{-2} s^{-1}$ during the observation period. There was no significant difference in soil respiration among treatments after the first snow manipulation (24 December 2014). CO_2 fluxes under Addition were 109% and 66% larger than Control after the second (4 February 2015) and third (26 February 2015) snow manipulations, respectively ($P < 0.05$). However, no significant difference was observed subsequently. CO_2 fluxes under Reduction and under Control had no significant difference during the whole experimental period. In addition, cumulative winter and annual CO_2 fluxes was 27.3 ± 1.1, 26.5 ± 2.1, and 29.5 ± 1.3 g C m^{-2} and 480 ± 26, 480 ± 27, and 486 ± 30 g C m^{-2}, respectively, under Reduction, Control, Addition. There were no significant differences in cumulative winter and annual CO_2 fluxes among treatments (Fig. 3). The contributions of winter CO_2 fluxes to annual CO_2 fluxes were 5.7 ± 0.2%, 5.5 ± 0.3%, and 5.8 ± 0.1% under Reduction, Control, and Addition, respectively. Q_{10} values during the winter were 13.3 ± 1.4, 14.6 ± 1.6, and 23.2 ± 2.0 under Reduction, Control, and Addition, respectively (Fig. 3). The statistical analysis showed that Q_{10} value under Addition was significantly higher than that under Reduction and Control ($P < 0.05$), but no difference was found between Reduction and Control.

3.3. Soil inorganic N and extractable organic C and N

The concentrations of soil ammonium, nitrate, EON and EOC ranged from 5.76 to 14.5 mg kg^{-1}, 20.1 to 32.5 mg kg^{-1}, 10.5 to 23.0 mg kg^{-1}

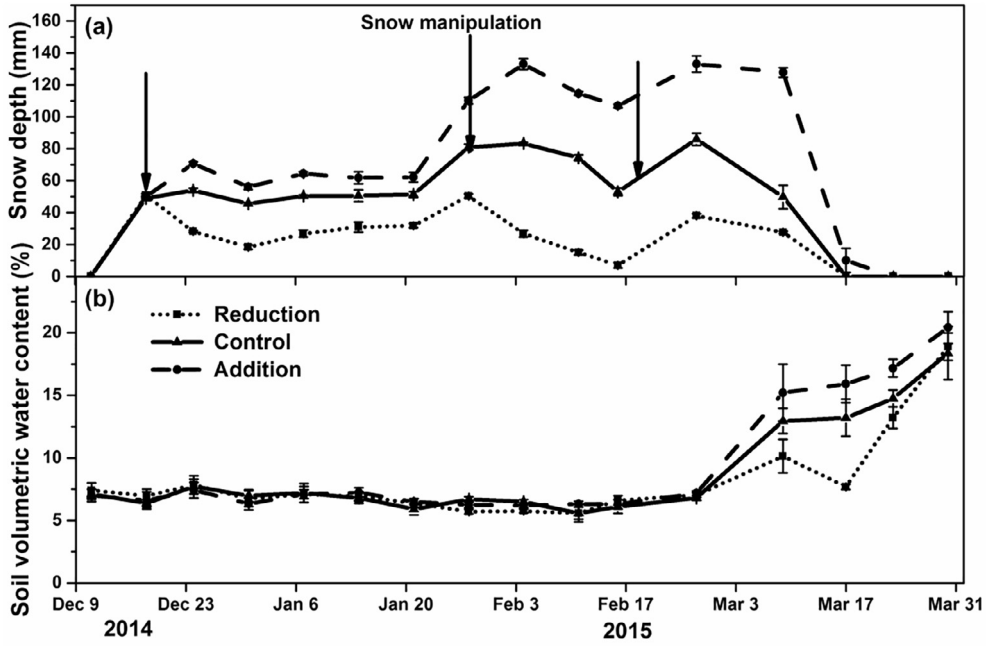

Fig. 1. Temporal variations of snow depth (a) and soil volumetric water content (b) among treatments. The vertical bars stand for standard errors (n = 5). Arrows indicate the date of snow manipulations.

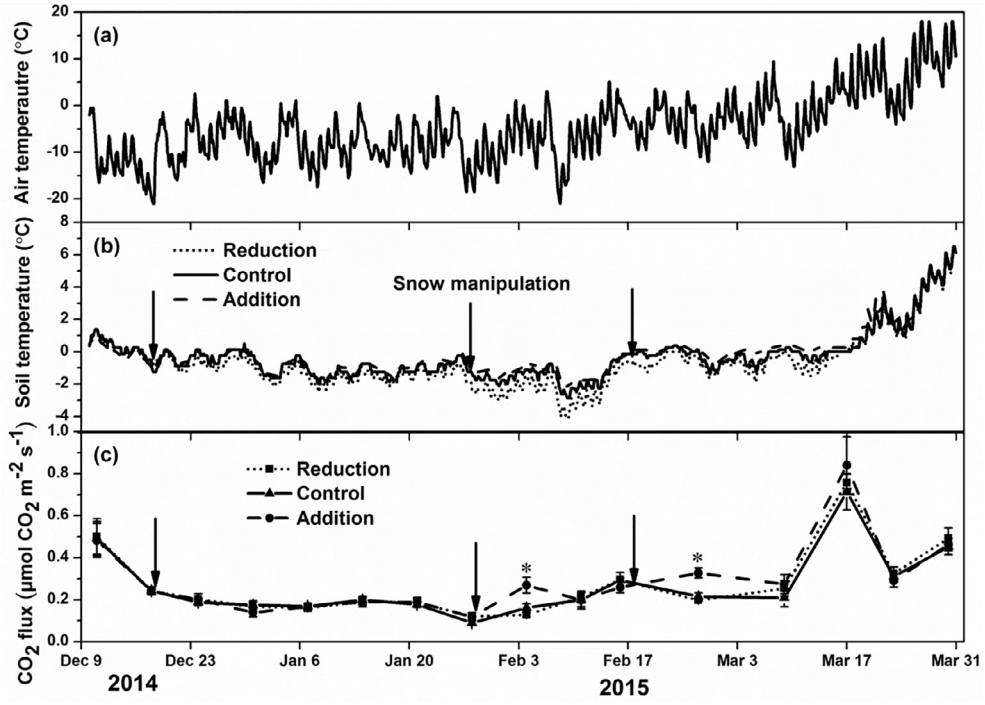

Fig. 2. Daily mean air temperature at 2 m aboveground (a) and effects of altered snow depths on soil temperature at 5 cm depth (b) and winter soil respiration (c) under snow reduction (short dot line), control (solid line) and addition (dash line) treatments. Arrows indicate the date of snow manipulation. The vertical bars stand for standard errors (n = 5). Asterisk represents significant difference between control and snow addition treatment ($P < 0.05$).

and 280 to 329 mg kg^{-1}, respectively. Snow cover did not affect soil ammonium, nitrate, EON and EOC during the whole observation period (Supplementary Fig. 1).

3.4. Soil microbial biomass C and N

Soil MBC and MBN and the ratio between them varied with time, showing higher values during the snow melt period than during the snow cover period (Table 1). The snow manipulation did not change MBC and MBN at the first shoveling but changed after the second and third snow manipulations. Snow addition enhanced MBC and MBN by 36% and 97% after the second snow manipulation and by 26% and 37% after the third snow manipulation, respectively ($P < 0.05$). However, these differences disappeared at the second sampling 7 days after both manipulations (Table 1). MBC:MBN was not affected significantly by the snow manipulation. Post-hoc analysis indicated that the significant differences in soil MBC and MBN existed between Addition and Control but not between Reduction and Control.

3.5. Soil microbial community structure

Soil microbial communities were remarkably affected by altered snow depths during the whole experimental period. There were no differences among treatments in the abundances of G^+, G^-, total bacteria, actinomycete, total fungi and AM fungi after the first snow manipulation (Supplementary Fig. 2). However, these parameters were all pronouncedly higher under Addition than under Reduction and Control 7 days after the second and third snow manipulations ($P < 0.05$). Then, no notable difference was found at the following observation. The abundances of G^+, G^-, bacterial, actinomycete, fungi and AM fungi under Reduction were not significantly different compared to that under Control.

PLFA analysis indicated that altered snow depths significantly affected the structure of soil microbial communities (Fig. 4). In the principal component analysis (PCA) of PLFA, snow addition significantly altered the loadings the second axis (PC2) after the second snow manipulation ($P < 0.05$, Fig. 4b), but not at the sampling one week after the second snow manipulation (Fig. 4c). A similar pattern was observed at the third snow manipulation. There was no difference in the structure of soil microbial communities after the first snow manipulation (Fig. 4a). The F/B under Addition was significantly higher than that under Control and Reduction after the second and third snow manipulations ($P < 0.05$, Table 1), but G^+/G^- had no apparent difference among different treatments (Table 1). Again, no marked difference was found after the first snow manipulation and at the sampling one week after the second and third snow manipulations (Table 1).

RDA indicated that PLFA abundances were explained actually by soil temperature ($P < 0.001$), and also by soil MBC ($P = 0.047$, Fig. 5). Other factors did not correlate with soil microbial community structure.

3.6. Soil extracellular enzymatic activities

Snow manipulations had a similar effect on extracellular enzyme activity compared to PLFA. Snow manipulation affected the activity of β-glucosidase but not that of α-glucosidase, cellobiohydrolase and xylosidase. Under snow addition, β-glucosidase activity was 53% and 40% higher than that under the two lower snow depths after the second and third snow manipulations ($P < 0.05$). However, there was no difference after the first snow manipulation, at the second observation after the second and third snow manipulations (Table 1).

3.7. Relationships between soil respiration and soil characteristics

Pearson's correlation analysis showed that soil temperature was positively correlated with air temperature ($r = 0.48$) and snow depth ($r = 0.66$). Winter soil CO_2 efflux was positively correlated with snow depth ($r = 0.73$), air temperature ($r = 0.62$), soil temperature ($r = 0.85$), MBC ($r = 0.82$), MBN ($r = 0.32$) and β-glucosidase ($r = 0.53$, Table 2). Among all abiotic variables, microbial C correlated most closely with soil temperature ($r = 0.75$).

Multiple stepwise regressions indicated that the relationship between soil CO_2 fluxes and MBC and EOC met the following equation: $Y = -0.724 + 0.02X_1 + 0.02X_2$, where Y: soil CO_2 fluxes; X_1: MBC; X_2: EOC. This equation explained 75.4% variations of winter soil respiration ($n = 75$, $P < 0.001$).

Table 1
Effects of snow depth manipulations on soil respiration, soil temperature, MBC[†], MBN[‡], MBC:MBN, G^+/G^-§, F/B[#], α-glucosidase, β-glucosidase, cellobiohydrolase, and xylosidase activities at the 6th day after the first snow manipulation (Snow man. 1), the 7th day after the second snow manipulation (Snow man. 2) and one week later, the 7th day after the third snow manipulation (Snow man. 3) and 8 days later, respectively. P-values of repeated measures ANOVA on the effects of snow depth manipulations and sampling time on these variables were shown in the last three rows.

			Soil respiration $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Soil temperature °C	MBC $mg\ kg^{-1}$	MBN $mg\ kg^{-1}$	MBC:MBN	G^+/G^-	F/B	α−glucosidase $nmol\ g^{-1}\ h^{-1}$	β−glucosidase $nmol\ g^{-1}\ h^{-1}$	Cellobiohydrolase $nmol\ g^{-1}\ h^{-1}$	Xylosidase $nmol\ g^{-1}\ h^{-1}$
Snow man.1	6 d	Reduction	0.16 ± 0.02	−0.51 ± 0.24	190.9 ± 18.0	14.9 ± 1.4	12.0 ± 1.1	1.19 ± 0.06	0.13 ± 0.01	2.1 ± 0.3	52.8 ± 4.7	11.3 ± 0.8	5.7 ± 0.7
		Control	0.17 ± 0.01	−0.10 ± 0.15	165.9 ± 8.3	15.6 ± 1.4	10.6 ± 0.9	1.18 ± 0.03	0.13 ± ± 0.01	2.2 ± 0.3	49.7 ± 4.7	12.1 ± 0.8	5.5 ± 0.6
		Addition	0.21 ± 0.05	−0.03 ± 0.19	186.3 ± 13.7	16.4 ± 2.1	10.0 ± 0.8	1.22 ± 0.06	0.12 ± 0.01	2.5 ± 0.5	51.5 ± 8.1	12.6 ± 0.4	5.6 ± 1.1
Snow man.2	7 d	Reduction	0.12 ± 0.01a	−1.69 ± 0.15a	151.4 ± 8.2a	16.8 ± 2.3a	9.7 ± 0.9	1.56 ± 0.09	0.13 ± 0.01a	2.0 ± 0.3	40.2 ± 2.7a	9.5 ± 0.6	5.0 ± 0.6
		Control	0.16 ± 0.02a	−1.14 ± 0.10b	167.2 ± 7.3a	14.3 ± 1.5a	11.7 ± 1.0	1.61 ± 0.11	0.14 ± 0.01a	2.0 ± 0.3	43.8 ± 3.9a	8.7 ± 1.4	4.9 ± 0.6
		Addition	0.28 ± 0.04b	−0.75 ± 0.11c	227.5 ± 30.3b	28.2 ± 3.6b	9.6 ± 1.5	1.50 ± 0.06	0.16 ± 0.01b	3.0 ± 0.6	67.0 ± 6.3b	10.7 ± 1.6	5.4 ± 0.6
	14 d	Reduction	0.21 ± 0.02	−2.84 ± 0.19a	178.2 ± 11.9	18.1 ± 3.4	11.6 ± 1.6	1.06 ± 0.07	0.13 ± 0.01	2.2 ± 0.6	56.2 ± 8.5	11.0 ± 4.2	5.2 ± 0.6
		Control	0.20 ± 0.03	−1.96 ± 0.16b	212.5 ± 28.9	22.5 ± 2.7	13.6 ± 1.4	1.01 ± 0.05	0.14 ± 0.02	2.4 ± 0.1	58.3 ± 4.6	10.2 ± 1.9	6.0 ± 0.5
		Addition	0.19 ± 0.04	−1.80 ± 0.12b	187.4 ± 29.9	22.9 ± 6.3	14.9 ± 2.5	1.18 ± 0.09	0.14 ± 0.01	2.0 ± 0.4	60.4 ± 3.2	10.6 ± 2.5	6.2 ± 0.5
Snow man.3	7 d	Reduction	0.23 ± 0.03a	−0.73 ± 0.18a	152.1 ± 17.2a	12.7 ± 1.6a	12.7 ± 0.6	1.13 ± 0.03	0.13 ± 0.01a	2.8 ± 0.3	46.0 ± 4.2a	8.7 ± 2.0	6.6 ± 0.6
		Control	0.23 ± 0.02a	−0.21 ± 0.06b	150.5 ± 15.1a	13.5 ± 1.8a	11.4 ± 1.1	1.14 ± 0.05	0.13 ± 0a	2.5 ± 0.5	49.8 ± 4.8a	8.1 ± 1.6	6.4 ± 0.9
		Addition	0.33 ± 0.02b	0.02 ± 0.03c	189.8 ± 3.9b	18.4 ± 2.3b	11.8 ± 0.9	1.20 ± 0.10	0.15 ± 0b	2.2 ± 0.4	69.8 ± 1.1b	7.3 ± 1.3	5.1 ± 0.4
	15 d	Reduction	0.26 ± 0.04	−0.14 ± 0.07a	291.7 ± 38.2a	20.4 ± 4.9	17.8 ± 2.6	1.13 ± 0.04	0.13 ± 0	1.5 ± 0.2	42.6 ± 7.3	7.2 ± 0.9	4.9 ± 1.9
		Control	0.21 ± 0.04	0.26 ± 0.11b	356 ± 47.6ab	26.1 ± 2.4	16.9 ± 1.1	1.01 ± 0.03	0.13 ± 0	1.5 ± 0.3	44.0 ± 4.5	8.2 ± 1.1	6.4 ± 0.7
		Addition	0.28 ± 0.05	0.50 ± 0.16b	371.8 ± 18.9b	22.2 ± 3.4	19.0 ± 2.4	1.13 ± 0.03	0.13 ± 0.02	1.8 ± 0.3	52.3 ± 6.5	8.8 ± 1.0	5.0 ± 0.3
Time			0.07	$< 0.01^{**}$	$< 0.01^{**}$	0.05^{*}	$< 0.01^{**}$	$< 0.01^{**}$	0.24	0.04^{*}	0.1	$< 0.01^{**}$	0.67
Treatment			0.02^{*}	$< 0.01^{**}$	0.03^{*}	$< 0.01^{**}$	0.96	0.32	0.48	0.74	$< 0.01^{**}$	0.77	0.75
Time * Treatment			0.27	0.02^{*}	0.03^{*}	0.27	0.45	0.63	0.66	0.48	0.23	0.83	0.74

Values were reported as mean ± standard errors. Different lowercase letters denote significant differences ($P < 0.05$) among treatment at each period. No letters indicate no significant differences among treatment at the period. ** highly significant ($p < 0.01$); * significant ($p < 0.05$).
[†]MBC: microbial biomass carbon.
[‡]MBN: microbial biomass nitrogen.
§G^+/G^-: ratio of gram positive bacteria to gram negative bacteria.
#F/B: ratio of fungi to bacteria.

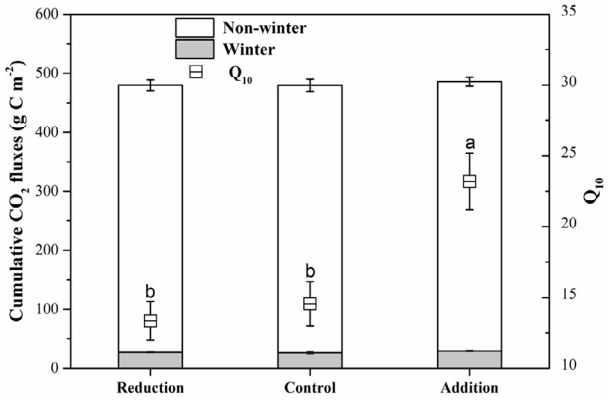

Fig. 3. Effects of snow depth manipulation on cumulative winter CO_2 fluxes, non-winter CO_2 fluxes, and Q_{10} (Q_{10}: increased soil respiration rate per 10 °C increase in soil temperature). The vertical bars stand for standard errors (n = 5). Different lowercase letters denote significant differences at $P < 0.05$. No letter indicates non-significant differences.

4. Discussion

Although the mean winter CO_2 effluxes ($0.32 \pm 0.07\ \mu mol\ m^{-2}\ s^{-1}$, Fig. 2c) of our studied forest plantation ecosystem in the mid-latitude region was lower than the previously reported values in Austria ($0.64\ \mu mol\ m^{-2}\ s^{-1}$, Schindlbacher et al., 2007) and in Washington State, USA ($0.67\ \mu mol\ m^{-2}\ s^{-1}$, McDowell et al., 2000), the contribution of wintertime soil CO_2 effluxes to annual soil CO_2 effluxes (5.5–5.8%) in our study was similar to the reported values (3.5–7.3%) from the mid-latitude ecosystems (Wang et al., 2010; Shi et al., 2012). The contribution of wintertime soil CO_2 effluxes to annual soil CO_2 effluxes was lower than the reported values (8–50%) from the high-latitude and alpine ecosystems (Kurganova et al., 2003; Vogel and Valentine, 2005; Schindlbacher et al., 2007) due to the shorter winter time and thinner snow depth in mid-latitude ecosystems than in high-latitude and alpine ecosystems.

A transient response of winter soil respiration to increased snow depth was found based on our one-year observation results. When snow depth was manipulated after the first snow manipulation, snow addition or reduction treatments had no significant effect on winter soil respiration, whereas when snow depth was manipulated at the 7th day after the second and third manipulations, winter soil respiration significantly increased under snow addition treatment compared to under control, but only for a few days. In contrast, although snow reduction reduced soil temperature on both the 7th and the 14th days after treatment, soil respiration did not change (Table 1). Previous studies in high latitude or altitude ecosystems with thicker snow depths have all observed a consistent stimulation of winter soil respiration with increasing snow depth and a decline of soil respiration with decreasing snow depth (Brooks et al., 1997; Monson et al., 2006b; Elberling, 2007; Nobrega and Grogan, 2007; Sullivan et al., 2008; Liptzin et al., 2009; Blankinship and Hart, 2012). While in our study, snow depth was much thinner than that in these previous studies. Meanwhile, the snow depth and microclimate in our study is rather typical for the continental climate with low winter precipitation in mid temperate areas of Northern China (Peng et al., 2010). We thus, conclude that increases in snowfall under these climatic conditions will only have a small and short-lived effect on soil respiration as the insulation effect is too small to persistently enhance soil respiration. The decreases in snowfall will have no significant effect on soil respiration, probably because soil microbes were adapted to the cold conditions under −1 °C and the decrease in temperature caused by snow reduction was small. Currently, the annual snow depth is ~6 cm on average in our study area (Chen et al., 2008), suggesting soils are probably frozen when air temperature gets below −10 °C. Therefore, warmer air temperature would play a more important role in soil respiration in the early spring due to its effect on the freeze-thaw cycles than in winter time. However, the close correlation between soil respiration and soil temperature and the observed high temperature dependency of soil respiration with Q_{10} values of 13.3–23.2 strongly suggest that a substantially greater increase in snow cover insulating soils effectively to temperatures above 0 °C would have dramatic effects on soil respiration during winter in these ecosystems.

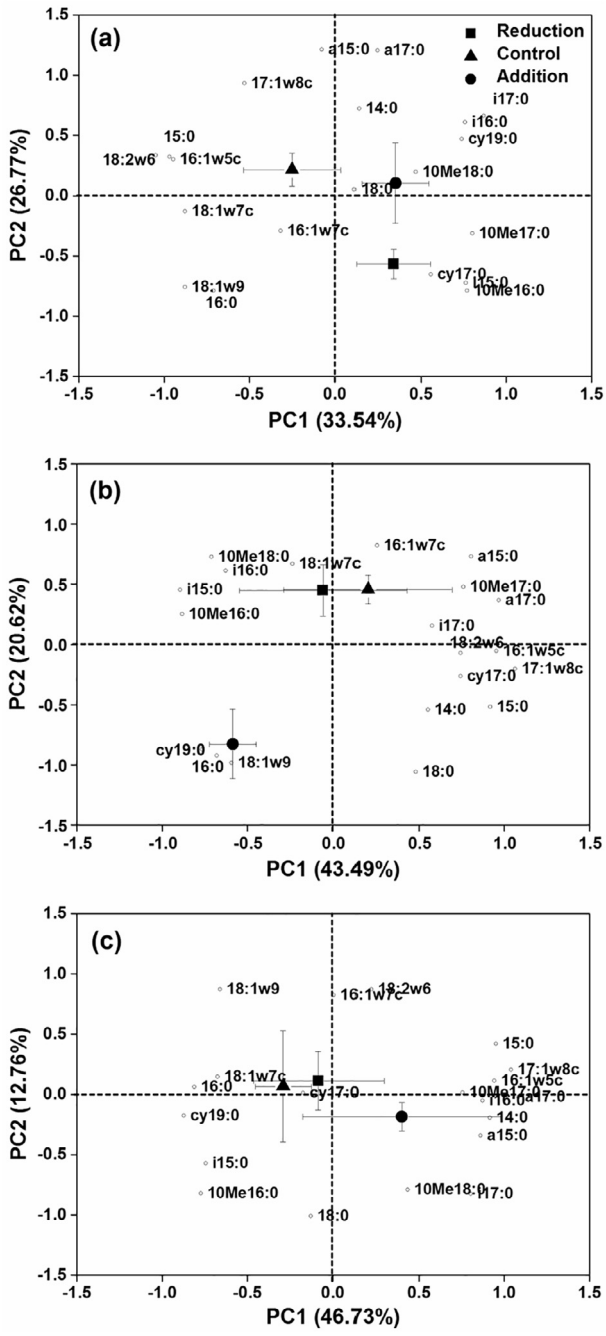

Fig. 4. PCA (PCA: principal component analysis) of soil microbial community composition under different snow depth manipulations at the 6th day after the first snow manipulation (a), the 7th day after the second snow manipulation (b) and one week later (c).

Soil temperature drives soil microbial enzymatic activities, which drive soil CO_2 efflux (Schindlbacher et al., 2007; Ruehr and Buchmann, 2010; Puissant et al., 2015). Snow depths influence soil temperature and moisture, the key regulators of soil microbial characteristics (Li et al., 2008; Aanderud et al., 2013; Shi et al., 2014; Tan et al., 2014),

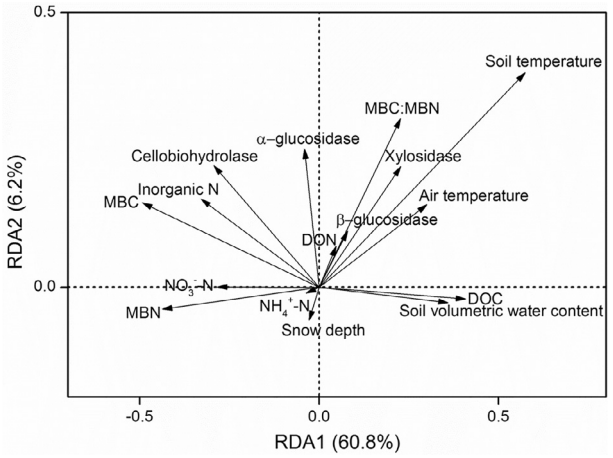

Fig. 5. RDA (‡‡ RDA: redundancy analysis)‡‡ of soil microbial community structure and environmental variables under different snow depth manipulations. Two axes (RDA1 and RDA2) account for 60.8% and 6.2% of total RDA variance, respectively.

and change soil nutrient conditions (Campbell et al., 2014; Tan et al., 2014) due to the changes of fine root activity or biomass (Koponen et al., 2006; Comerford et al., 2013; Campbell et al., 2014; Reinmann and Templer, 2016) and the release of dissolved organic matter from fresh litter and soil aggregates (Steinweg et al., 2008; Wu et al., 2010; Freppaz et al., 2012). However, we found none of soil temperature, soil water content, soil nitrogen content, and soil microbial characteristics (including microbial biomass, microbial community structure, and extracellular enzymatic activities) were altered by snow addition after the first snow manipulation, which is in agreement with the unchanged soil respiration under the same treatment. This was probably because a small increase in snow depth could not increase the insulation effect and had no significant effect on soil temperature, leading to unchanged soil microbial characteristics. By comparison, when soil temperature significantly increased at the 7th day following the 2nd and 3rd snow manipulation events, although soil nutrient and soil water content did not significantly change, soil microbial biomass carbon and nitrogen and β-glucosidase activity all significantly enhanced and microbial community structure (F/B) also evidently changed denoted by the increased F/B based on our one-year observation results. The fungi could exploit water and nutrients from deeper soils using hyphae and decompose complex and polymeric carbon compounds (Aanderud et al., 2013), leading to faster growth in fungal community than in bacterial community during the cold winter, which may be the reason for the increase in F/B (Table 1). Correlation analysis indicated that the observed significantly increase in soil temperatures due to a thicker snow cover was responsible for the increased soil respiration, microbial biomass carbon and nitrogen, and β-glucosidase activity (Table 2, Supplementary Fig. 3). In agreement, redundancy analysis of PLFA showed significant positive relationship between soil temperature and microbial community structure (Fig. 5), which is consistent with previous results (Robroek et al., 2013). Therefore, although our treatment experiment may cause disturbance to the system, we believe the observed increase in soil respiration after treatments were the results of increased snow cover and temperature. Interestingly, the snow reduction had no effect on soil respiration, water content, nutrients, or soil microbial characteristics, although the cooling effect due to snow reduction was much more pronounced than the small warming effect due to snow addition (Fig. 2b). This reflects that soil microbes responded to the little warming, but remained steady with stronger cooling, probably due to the quite quick acclimation of soil microbial communities to reduced soil temperature (less than 1.5 °C).

In agreement with soil respiration, the distinct augmentation in soil microbial biomass carbon and nitrogen, F/B, β-glucosidase activity, and

Table 2
Relationships between CO_2 flux and air temperature, soil temperature, soil moisture, snow depth, NO_3^--N, NH_4^+-N, EOC**, EON††, MBC‡‡, MBN#, α-glucosidase, β-glucosidase, cellobiohydrolase and xylosidase activities (n = 30).

	CO_2 flux	Snow depth	Air temperature	Soil temperature	Soil moisture	NO_3^--N	NH_4^+-N	EOC	EON	MBC	MBN	α-glucosidase	β-glucosidase	Cellobiohydrolase	Xylosidase
CO_2 flux	–														
Snow depth	0.734**	–													
Air temperature	0.617**	0.504**	–												
Soil temperature	0.848**	0.660**	0.479**	–											
Soil moisture	0.087	0.092	0.081	−0.021	–										
NO_3^--N	0.221	0.056	0.037	0.031	−0.040	–									
NH_4^+-N	0.060	0.043	0.270	0.026	−0.081	0.441*	–								
EOC	0.159	0.280	0.132	0.023	0.292	0.257	0.335	–							
EON	0.252	−0.045	0.020	−0.056	0.183	−0.482*	−0.324	0.164	–						
MBC	0.818**	0.568**	0.445*	0.750**	0.086	−0.108	−0.231	−0.238	0.053	–					
MBN	0.324*	0.359*	0.321	0.294*	0.206	−0.250	−0.339	−0.233	0.108	0.575**	–				
α-glucosidase	−0.116	−0.144	0.073	0.147	0.036	0.187	0.028	0.032	−0.009	−0.291	−0.256	–			
β-glucosidase	0.528**	0.375*	0.350*	0.414*	−0.055	0.193	−0.190	−0.075	0.237	0.430*	0.346	0.145	–		
Cellobiohydrolase	−0.186	−0.289	−0.257	−0.106	−0.303	−0.164	−0.013	0.016	0.320	0.143	0.089	0.408*	0.314	–	
Xylosidase	−0.166	−0.222	0.156	0.056	0.086	−0.051	0.046	0.233	0.027	0.070	0.047	0.495**	−0.203	0.196	–

** highly significant ($P < 0.01$). * significant ($P < 0.05$).
**EOC: extractable organic carbon.
††EON: extractable organic nitrogen.
‡‡MBC: microbial biomass carbon.
#MBN: microbial biomass nitrogen.

soil temperature were only transient and lasted only a few days after the snow addition treatment. These results suggested the transient differences in soil temperature due to snow addition lead to the transient responses of soil microbial communities that disappeared once the difference in soil temperature disappeared. Finally, despite the short-lived increase in soil respiration after snow addition, there was no significant snow cover effect on cumulative winter soil respiration, suggesting that altered snow depth in mid-latitude regions may not cause a significant increase in soil respiration. Our conclusion is in agreement with the multi-year monitoring of soil respiration of Schindlbacher et al. (2014), who found that annual soil CO_2 effluxes were not affected by natural variations of snow cover in a mid-elevation temperate forest soil. In contrast, however, many studies conducted in areas with thicker snow depth and colder climate showed an increase of cumulative winter soil respirations under snow addition treatments (Elberling, 2007; Nobrega and Grogan, 2007; Liptzin et al., 2009).

5. Conclusions

In continental temperate China with low winter precipitation, we found a transient response of winter soil respiration to increased snow depth. Our one-year observation results suggest that if the mean annual snow depth increases from the current 6 cm to 12 cm in the future, it probably would not change annual or winter cumulative CO_2 effluxes in this study area. However, the increase of snow depth caused significant, but transient increases in soil temperature (< 1 °C), microbial biomass, enzyme activities, and therefore soil respiration. These results suggested a transient response of soil microorganisms due to the transient difference in soil temperature and a short-lived increase in soil respiration, which did not significantly affect annual soil CO_2 effluxes. However, due to the high temperature sensitivity of soil respiration and the tight correlation between soil temperature and respiration, we should still remain vigilant to the predicted future increase in snow cover in this area, especially when it increases soil temperature to above 0 °C.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2018.05.010.

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